Auxin transport-feedback models of patterning in plants

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ABSTRACT

Many patterning events in plants are regulated by the phytohormone auxin. In fact, so many things are under the influence of auxin that it seems difficult to understand how a single hormone can do so much. Auxin moves throughout the plant via a network of specialized membrane-bound import and export proteins, which are often differentially expressed and polarized depending on tissue type. Here, we review simulation models of pattern formation that are based on the control of these transporters by auxin itself. In these transport-feedback models, diversity in patterning comes not from the addition of more morphogens, but rather by varying the mechanism that regulates the transporters.

Key-words: auxin; canalization; modelling; morphogenesis; patterning; phyllotaxis; transport; venation.

AUXIN DRIVES PATTERNING IN PLANTS

From the establishment of the first embryonic axis to the formation of leaves and flowers in adult plants, many patterning events in plant development involve the phytohormone auxin (Benkova *et al.* 2003; Friml *et al.* 2003; Reinhardt *et al.* 2003; Blilou *et al.* 2005; Tanaka *et al.* 2006). In the embryo, plants must initially define the apical/basal axis to correctly position the shoot apical meristem at one end and the root apical meristem at the other. Disrupting auxin transport by mutations or inhibitor treatments compromises this early patterning process, resulting in unviable embryos (Steinmann *et al.* 1999; Friml *et al.* 2003; Vieten *et al.* 2005; Weijers *et al.* 2005, 2006; Jenik, Gillmor & Lukowitz 2007). As the shoot apex develops, plants use auxin to position the organs, leaves or flowers, at regular angles around the stem axis, resulting in patterns called phyllotaxis (Reinhardt *et al.* 2003; Heisler *et al.* 2005; Kuhlemeier 2007). Equally important is the formation of the vascular system to deliver nutrients, water and photo assimilates throughout the plant. Although the patterns of interconnected veins seen in leaves (Fig. 1B) look very different from phyllotactic patterns (Fig. 1A), vein specification is thought to be driven by auxin (Sachs 1969, 1975, 1981; Scarpella & Meijer 2004; Scarpella *et al.* 2006). Auxin

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is also involved in controlling the formation of lateral roots, which originate post-embryonically, by promoting the recruitment of lateral root primordium founder cells from the pericycle tissue (Casimiro *et al.* 2001; Benkova *et al.* 2003; De Smet *et al.* 2007; Ditengou *et al.* 2008; Dubrovsky *et al.* 2008; Swarup *et al.* 2008). At a larger scale, auxin is involved in controlling the relatively long distance signalling processes that result in apical dominance (Ongaro & Leyser 2008). The plant hormone has also been shown to trigger major developmental reconfiguration events in response to external developmental cues such as tropic responses of the plant to light and gravity (Marchant *et al.* 1999; Friml *et al.* 2002; Tatematsu *et al.* 2004; Swarup *et al.* 2005; Esmon *et al.* 2006).

At the core of all these patterning processes is the establishment of auxin gradients (Benkova *et al.* 2003). The visualization of these auxin gradients allows us to predict where tissue differentiation will occur. For instance, GFP (green fluorescent protein) expression driven by the synthetic auxin responsive promoter DR5 appears in the surface of the shoot apical meristem at the site of organ formation well ahead of any physical signs of organogenesis (Heisler *et al.* 2005; Smith *et al.* 2006a). Similarly, the formation of strands high in DR5 reporter expression in young leaves defines where the vein network will eventually differentiate, nearly a day before the expression of early procambium markers such as ATHB8 (Scarpella *et al.* 2006). Although auxin accumulation at specific sites can induce differentiation, the activation of a certain developmental pathway also depends on additional tissue-specific fate specification factors. For instance the micro-application of auxin in the peripheral zone of the shoot meristem triggers organogenesis whereas application to young leaves induces the formation of additional vascular strands (Reinhardt, Mandel & Kuhlemeier 2000; Scarpella *et al.* 2006). As Ottoline Leyser writes, 'When the auxin baton points your way, it's your turn to play whatever musical instrument you happen to be holding' (Leyser 2005).

If the auxin gradients are indeed the main driving force behind many patterning events in plants, then it is natural to ask how these auxin gradients are established, given they often must start *de novo*. An example of this *de novo* patterning capability can be seen in the regeneration of an entire plant from a single cell isolated from leaf mesophyll (Takebe 1971). As this single cell grows and divides, its progeny must collectively decide which cells will become shoot, and which will become root. These cells all have the same genetic code, but somehow, they are able to
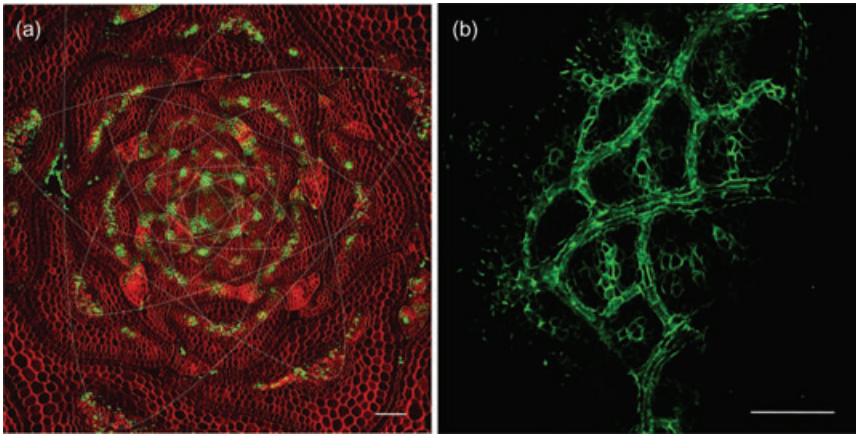
Figure 1. Examples of auxin-induced patterning in plants. (a) Transversal section through Arabidopsis vegetative meristem showing spiral phyllotactic pattern. Green signal is the auxin exporter PIN1 and red signal is calcofluor staining of the cell walls. (b) PIN1 (green) immunolocalization of young Arabidopsis leaf showing developing vein network. Scale bars, 50 μm.

communicate and self-organize to create the various structures and cell types required in the adult plant. Although not quite as dramatic, similar processes occur during venation in developing leaves and organ primordium initiation in the shoot apex, when a subset of cells is selected from a ground cell population, and subsequently undergoes specific differentiation.

TURING'S REACTION–DIFFUSION MODEL CAN CREATE *DE NOVO* PATTERNS

The question of how a group of equivalent cells self-organize to create a pattern was addressed by Turing (1952), and his reaction–diffusion model has since become one of the most widely used models for pattern formation in biology (Meinhardt 1982; Murray 2002). Turing's model fits nicely within the modern framework of genetic regulatory networks, which are often modelled with differential equations for the production, decay and interaction of gene products. This was one aspect of Turing's model, the reactions of substances he called morphogens, and the feedbacks and interactions between them. However, Turing was interested in pattern formation, and thus his theory also included a spatial component. Whereas genetic regulatory network modelling is often focused on the workings of a single cell, Turing considered a system of multiple cells,[1] where the output from the reaction network in one cell becomes the input to the reaction network in the next. The cell-to-cell communication was in the form of diffusion, hence the name *reaction–diffusion*.

To see how reaction–diffusion can create a pattern, it is instructive to look at the *activator–inhibitor* model developed by Gierer and Meinhardt (1972). They proposed that patterning requires the cooperation of two components: *local activation*, to select a subset of cells for differentiation, combined with a *longer range inhibition*, to suppress the activation of neighbouring cells. In their model, a substance called the activator enhances its own production, as well as that of second substance, termed the inhibitor. The inhibitor

inhibits the production of the activator. This system can be seen as a simple genetic regulatory network operating in each cell. The cells communicate via diffusion of the two substances. A small local maximum in activator concentration in one cell because of random variation leads to a local increase in production of both the activator and the inhibitor. The inhibitor diffuses away more quickly than the activator, reducing its effect on local activator self-enhancement, while suppressing activator self-enhancement in neighbouring cells. In a system of identical cells, each operating with identical reaction rules, this destabilization can create a spatial pattern of peaks in activator concentration, which can trigger selective differentiation leading to patterning (Fig. 2A). By using interactions of multiple substances, combined with multiple cascading interactions, Meinhardt has used this basic idea to account for a wide variety of patterning processes observed in nature (Meinhardt 1982, 1995), including the appearance and interpretation of morphogen gradients (Wolpert 1969). Much of Meinhardt's work was done before the widespread availability of molecular data about the morphogenetic substances involved. However, reaction–diffusion models are now appearing as genetic regulatory networks linked directly to identified gene products in both plants and animals (Jönsson *et al.* 2005; Sick *et al.* 2006; Bouyer *et al.* 2008; Digiuni *et al.* 2008).

AUXIN IS DIFFERENT FROM TURING'S MORPHOGENS

Although Turing's reaction–diffusion model can account for a wide variety of biological patterning and *de novo* pattern formation, auxin differs from the morphogens considered by Turing and Meinhardt in two important ways. First, the movement of auxin through plant tissue does not occur primarily by diffusion, but by polar transport. Auxin carrier proteins move auxin from cell to cell in ways diffusion cannot, for example, up a concentration gradient. Second, it appears that in many cases, auxin gradients that are established in plant tissue do not result from the local production of auxin, but rather by redistributing auxin from surrounding tissue via polar transport. It is thus the control

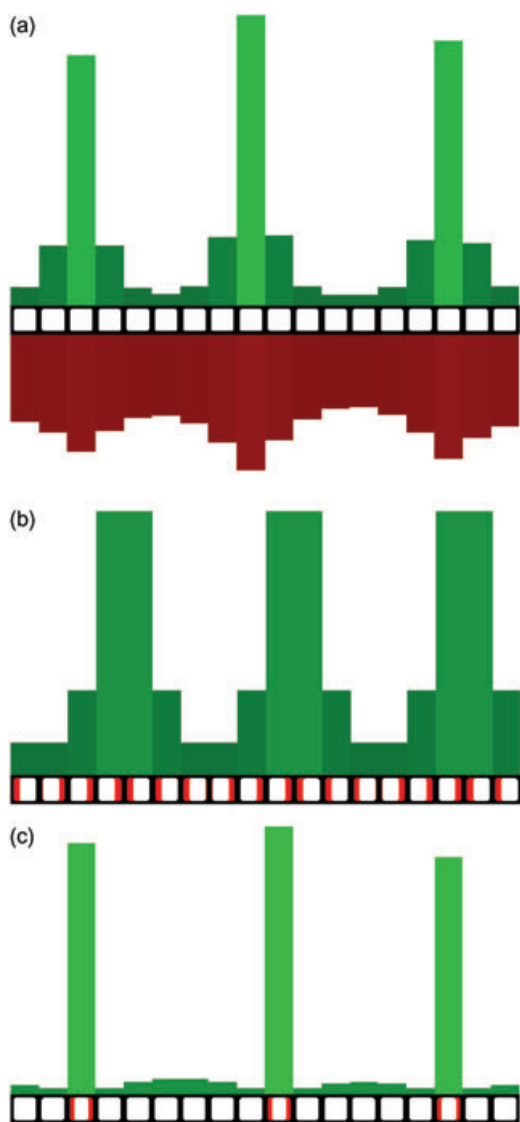[1]Turing also presented a continuous version of his theory based on PDEs.

Figure 2. Different patterning mechanisms can create similar patterns on a line of cells. (a) Turing style reaction-diffusion patterning based on Gierer & Meinhardt's (1972) activator-inhibitor system (activator shown in green, inhibitor shown in red). (b) Transport-feedback patterning based on up-gradient PIN1 orientation (auxin shown in green, PIN1 shown in red). (c) Transport-feedback patterning based on the upregulation of AUX1 (auxin shown in green, AUX1 shown in red). Simulations performed with wrap around boundary conditions. For simulation equations and parameters see the supplemental materials.

of the rate and the direction of auxin transport that is central to auxin-based patterning.

It has been known for some time that plants transport auxin in a polar fashion (Goldsmith 1966; Leopold & Hall 1966). To explain this phenomenon, the *chemiosmotic model* of auxin transport was developed (Rubery & Sheldrake 1974; Raven 1975; Goldsmith & Goldsmith 1981; Goldsmith, Goldsmith & Martin 1981). Auxin (indode-acetic-acid) is a weak acid, and in the neutral pH inside cells, it is largely

dissociated. In this ionic form, auxin is hydrophilic and unable to cross the plasma membrane. In order for auxin to leave a cell, it requires the activity of carriers located at the plasma membrane. One of these carriers, the PIN1 export protein in Arabidopsis, is so important in the shoot that the *pin1* mutant is unable to generate flowers, and produces a pin-shaped inflorescence instead (Okada *et al.* 1991). Once outside the cell, in the lower pH of the extracellular space, a significant portion (approximately 20%) of the auxin becomes protonated, making it lipophilic and able to cross the plasma membrane and re-enter cells passively. However, there is evidence that passive influx is not always enough for reliable patterning. Although the phenotypes are not as severe as the PIN1 mutant, Arabidopsis plants missing several import carriers show major disruption in organ positioning (Bainbridge *et al.* 2008). Figure 3 shows a schematic representation of how auxin is thought to move through cells. Import carriers located in the plasma membrane import auxin into the cytosol from extracellular space. Once inside a cell, auxin moves via diffusion, and is then transported out of the cell by export carriers. The export carriers are often polarly localized to one side of the cell, and when coordinated over multiple cells, this polarity results in a directional flux of auxin through the tissue.

Since auxin gradients are created by active transport, the patterning mechanism behind the establishment of these
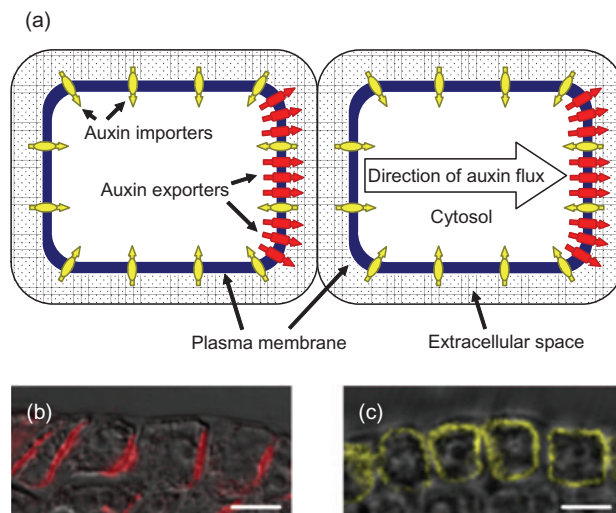

Figure 3. The polar transport of auxin through cells. (a) Schematic representation of auxin transport. From the extracellular space, the plant hormone auxin can enter cells passively or via auxin importers (yellow) located at the plasma membrane. Once inside the cell, auxin moves through the cytosol by diffusion and is then transported out of the cell by export carriers (red) which are often polarly localized to one side of the cell. The coordinated polar localization of the auxin exporters over multiple cells determines the overall direction of the auxin flux within tissue (adapted from Smith 2008). (b) Immunolocalization for PIN1 (red) in tomato shoot apex showing polarly localized exporters. (c) Immunolocalization for AUX1 (yellow) in Arabidopsis shoot apex showing the uniform non-polar localization of the importer at the plasma membrane (picture courtesy Katherine Bainbridge). Scale bars, 5 *μ*m.

gradients results from the control of the abundance and/or polarity of the auxin transporters at the plasma membrane. Experimental work suggests that auxin itself has a major effect on regulating its own transport, and that the asymmetric auxin distribution patterns seen in plant tissues are formed as a result of a feedback loop between auxin and its transporters. For example, it has been shown that auxin can regulate the expression of auxin export carriers (Heisler *et al.* 2005; Vieten *et al.* 2005). Auxin also modulates subcellular localization of its exporters, since Arabidopsis PIN1 and PIN2 proteins can re-localize upon auxin micro-application (Sauer *et al.* 2006; Bayer *et al.* 2009). High auxin levels inhibit endocytosis, locking export carriers at the plasma membrane, thus promoting auxin efflux (Paciorek *et al.* 2005). Moreover, the upregulation of the auxin importer AUX1 by auxin may help to establish an auxin maximum that triggers lateral root formation (Laskowski *et al.* 2008). This suggests that plants have found a variety of ways to use the feedback of auxin on its own transport to generate patterns, creating a class of *transport-feedback* patterning mechanisms.

A TRANSPORT-FEEDBACK MECHANISM FOR VEIN FORMATION: SACHS' CANALIZATION HYPOTHESIS

Sachs was perhaps the first to suggest that auxin could feed back on its own transport (Sachs 1969, 1975, 1981). By performing micro-application experiments on pea hypocotyls, he observed that auxin was sufficient to trigger the differentiation of vascular strands from the source (application site) to the intact central vein, which he believed was acting as a sink for auxin. The fact that such treatments led to the formation of narrow vascular strands could not be explained by diffusion alone, and he proposed the existence of a 'canalization' mechanism for auxin flux. He suggested that a plant cell's ability to transport auxin increases with auxin flux, and that this feedback of auxin on its own transport is responsible for selecting strands of tissue that later differentiate into vascular bundles. He drew the analogy with the way erosion leads to the formation of discrete channels with the flow of water. Any initially dominant path, however small, would undergo increased erosion, which would subsequently attract more flow. This would cause the path to enlarge (local activation), while simultaneously suppressing the formation of competing paths nearby (longer-range inhibition).

The involvement of auxin transport in vein formation has since been confirmed by complementary approaches. In mutants impaired in auxin transport machinery (Galweiler *et al.* 1998; Deyholos *et al.* 2000; Sieburth *et al.* 2006), auxin signalling (Przemeck *et al.* 1996; Hardtke & Berleth 1998; Hobbie *et al.* 2000) and auxin biosynthesis (Cheng, Dai & Zhao 2007), the leaf venation pattern is highly disrupted. A similar effect is seen when treating leaves with auxin transport inhibitors (Mattsson, Sung & Berleth 1999; Sieburth 1999; Mattsson, Ckurshumova & Berleth 2003). Moreover, GUS (beta-glucuronidase) or GFP expression driven by the auxin responsive promoter DR5 marks the initiating leaf vein (Mattsson *et al.* 2003; Scarpella, Francis & Berleth 2004; Scarpella *et al.* 2006) prior to the expression of the early pre-procambium marker ATHB8. This early DR5 reporter expression occurs concomitantly with the expression of the auxin exporter PIN1, and both are currently the earliest known markers for vascular initiation (Scarpella *et al.* 2006).

MITCHISON'S SIMULATION MODELS OF THE CANALIZATION

Mitchison explored the plausibility of Sach's canalization hypothesis with computer simulation studies (Mitchison 1980, 1981c). He proposed two main variants of the model, facilitated diffusion and polar transport, each suggesting different molecular mechanisms. In his facilitated diffusion model, the transporters were passive channels that simply increase the diffusion rate between cells. This increase is bidirectional, as each interface is symmetric, with flux in either direction causing an increase in the ability of auxin to move in both directions. Mitchison suggested plasmodesmata as potential candidates for these channels, although this hypothesis is lacking experimental support. In his polar transport model, the abundance of transporters at the cell periphery could vary on each side of the interface, and it was therefore necessary to consider the direction of flux when adding carriers. Only a net efflux of auxin from a cell across an interface induced the addition of carriers based on flux. If the efflux was less than zero (influx), then only a background amount of carriers was added. Mitchison showed that both models were able to produce discrete canals of auxin flow in a uniform field of cells that included a small amount of noise. He also reported that in order for the canalization of auxin to occur, the relationship between the addition of transporters (channels or carriers) and auxin flux must be non-linear, and used a quadratic function in his models. Feugier, Mochizuki & Iwasa (2005) confirmed this result when exploring Mitchison's polar transport model and tried various alternatives for the flux response function.

Rolland-Lagan & Prusinkiewicz (2005) performed simulations based on both the facilitated diffusion and the polar transport versions of Mitchison's model, comparing them with experimental data on vein formation in Arabidopsis leaves. They noted that the polar transport model was more consistent with the current theory of auxin transport (Fig. 3) and PIN1 expression data, in that it predicts a polarity of the carriers in the cells from source to sink. This can be seen during the initiation of the midvein, where the auxin exporter PIN1 displays an overall polarity directed away from the auxin maximum at the leaf tip towards the stem vasculature below (Reinhardt *et al.* 2003; Scarpella *et al.* 2006; Bayer *et al.* 2009). This process is repeated during the formation of the first secondary veins, where convergence points of PIN1 and DR5 reporter expression appear in the leaf margin and then extend into the leaf and connect to the midvein at the base of the leaf. Thus, as in the case of midvein initiation, PIN1 polarizes away from the source of auxin towards existing veins.

FINDING THE SINK

Sachs suggested that pre-existing vascular strands were acting as sinks for auxin, creating an auxin concentration gradient in the surrounding tissue, thereby attracting initiating veins towards them. Since the pre-existing vascular strands have high DR5 reporter expression and presumably high auxin concentration (Scarpella *et al.* 2006), it is not clear how they can act as a sink for auxin as suggested by Sachs. This raises the question how the developing vein is able to find pre-existing veins. Heisler & Jönsson (2006) suggest that these cells may still act as sinks if they express high levels of auxin import carriers, an idea supported by the simulation models of Kramer (2004). In order to explore the ability of Mitchison's models to find a sink, we performed simulations using the equations and parameters from Rolland-Lagan & Prusinkiewicz (2005) with both a point source of auxin, representing the convergence point at the leaf tip, and a point sink, representing the pre-existing vasculature. We found that the facilitated diffusion model could much more reliably connect the source to the sink than the polar transport model (Fig. 4). On a grid of cells, moving the sink just a few cells to the side causes the vein in the polar transport simulation to go past, and hit the end of the grid, before it turns and connects to the sink. We also found that the facilitated diffusion model is less sensitive to parameter values. With the polar transport model, too much carrier production or too much auxin in the system often creates small cycles of cells that pump auxin around in

circles. In the facilitated diffusion model, carriers can only transport auxin down its concentration gradient, and thus, cycles do not form. Sachs has observed such cycles experimentally after wounding, but notes that they are rare in intact plants (Sachs & Cohen 1982). Despite some attractive attributes, the facilitated diffusion model does not fit well with the experimental data, showing that auxin movement in plant tissue is polar.

In their simulation of midvein formation with a variation of the canalization model, Bayer *et al.* (2009) also found that the initiating vein could not reliably find the sink. However, experimental results strongly support the view that existing vasculature guides initiating veins to connect to it (Sachs 1981; Bayer *et al.* 2009). Because the sink effect of existing vasculature does not seem to be enough to attract the initiating veins, especially if the latter are high in auxin, they included an additional vein-attracting factor in their model. Although the biological nature of such a signal is unknown, Bayer *et al.* (2009) modelled it with a substance produced in existing veins that diffused through the meristem creating a gradient.

HIGH FLUX OR HIGH CONCENTRATION?

A long-standing criticism of Mitchison's model is that it predicts high flux, but low concentration in emerging veins. This seems in contradiction with experimental evidence that suggests that auxin concentration is high in developing veins. Vascular strands can be induced when exposed to
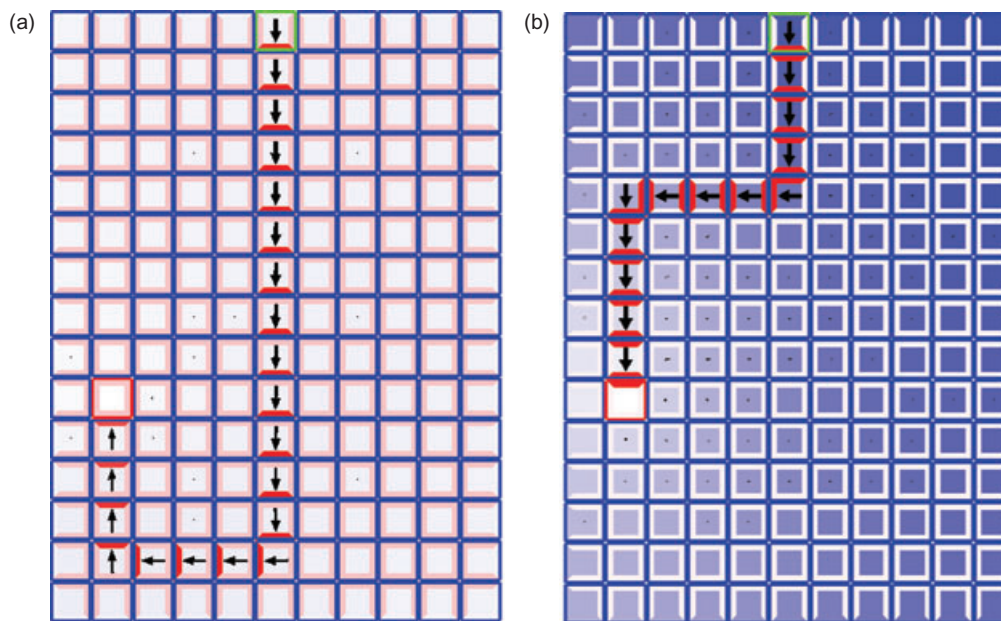

Figure 4. Comparing Mitchison's canalization models' ability to find a sink. Simulations of Mitchison's polar transport (a) and facilitated diffusion (b) models of canalization (adapted from Rolland-Lagan & Prusinkiewicz 2005). Blue indicates auxin concentration, red indicates carrier density at the cell membrane sections, and the black arrows indicate the strength and prominent direction of auxin flux. If an auxin sink (outlined in red) is placed immediately below an auxin source (outlined in green) both models will make a direct connection from source to sink. However, if the sink is moved just a few cells to one side, the strand in the polar transport model (a) extends past the sink, whereas the facilitated diffusion model (b) is able to find the more direct route. For simulation equations and parameters see the supplemental materials.
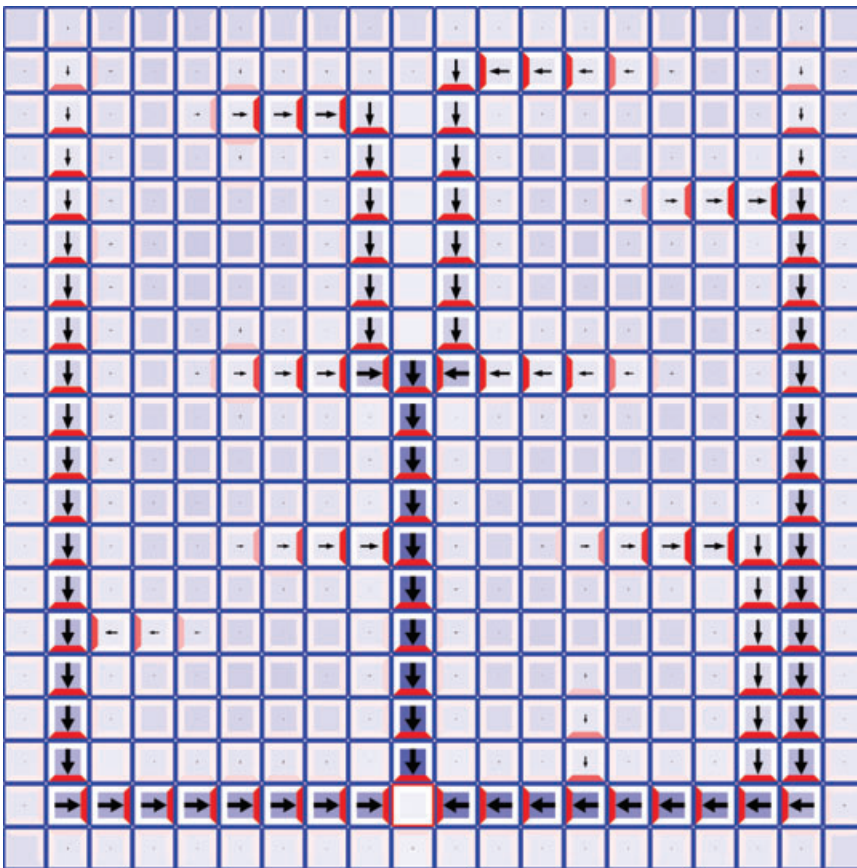
Figure 5. Feugier's polar transport model of the canalization of auxin flux based on the allocation of PIN (red) to cell membrane sections from a pool of PIN in the cytosol. Blue indicates auxin concentration, black arrows indicate the direction and magnitude of auxin flux. Note the development of high auxin concentration in collector veins. For simulation equations and parameters see the supplemental materials.

ectopic auxin micro-application (Sachs 1981; Sauer *et al.* 2006), and several auxin-induced genes are upregulated in initiating veins (Mattsson *et al.* 2003; Scarpella *et al.* 2004, 2006). Feugier addressed this problem by exploring different ways of allocating the carriers to the cell periphery. If carriers were allocated from a fixed pool in each cell, the saturating effect of limited carriers led to auxin accumulation in the veins compared to surrounding tissue (Fig. 5) (Feugier *et al.* 2005; Fujita & Mochizuki 2006a,b). In Mitchison's model, carriers were added to each wall independently, with the amount of carriers added at an interface only depending on the flux across that interface. The allocation of carriers from a pool is in agreement with experimental data showing that auxin carriers cycle between the plasma membrane and the endosomal compartment and that this cycling is important for dynamic polarization (Steinmann *et al.* 1999; Geldner *et al.* 2001; Geldner *et al.* 2003; Dhonukshe *et al.* 2007, 2008; Kleine-Vehn *et al.* 2008). However in Feugier's model, carrier (PIN1) expression domains initiate from a discrete sink cell at the base of the leaf, and are initially low in auxin. High concentration emerges after the canals become collectors for larger areas, supplied by smaller, feeder canals (Fig. 5). Note that high DR5::GFP signal is observed in Arabidopsis at the earliest stages of vein initiation, when the PIN1 expression domains are first forming (Scarpella *et al.* 2006; Bayer *et al.* 2009), which suggests that the PIN1 expression itself is in response to high auxin levels. Kramer (2004) proposed that the

presence of auxin importers may also be required to maintain a high concentration in developing veins.

MAKING LOOPS

The canalization hypothesis can account for the formation of open vein-branching patterns; however, many species, including Arabidopsis, display reticulate venation. After the primary midvein has formed, higher-order secondary veins create loops by connecting back to the primary midvein. During this initial loop formation, cells with bipolar PIN1 expression were found within the loop in young Arabidopsis leaves (Scarpella *et al.* 2006). These bipolar cells occur at the point where PIN1 polarity switches direction, suggesting that the upper part of the loop directs auxin towards the connection at the top of the midvein, with the lower portion draining towards the connection with the midvein at the base of the leaf. Heisler & Jönsson (2006) noted that this is consistent with the canalization model if this bipolar cell was a source of auxin, although Scarpella *et al.* (2006) did not report an upregulation of DR5 reporter expression at the bipolar cell. Exploring this aspect of vein patterning, Rolland-Lagan & Prusinkiewicz (2005) were able to create loops by including discrete auxin sources, and in some cases moving them as veins form. A role for discrete auxin sources is also supported by the more abstract model of Runions *et al.* 2005, which was able to generate highly realistic reticulated venation patterns. Feugier took a different approach,

creating loops by adding an additional factor to his model, and did not assume discrete auxin sources (Feugier & Iwasa 2006). Another possibility was suggested by Sachs; loops in venation might be the result of alternating directions of auxin flow. He induced loop formation in pea internodes by alternating the application of auxin to two different sites over a period of 5 d (Sachs 1975). This idea, however, does not easily fit with our current understanding of directional auxin transport, and would be much easier to reconcile with a facilitated diffusion model of canalization.

A transport-feedback model closely related to the canalization model for venation was proposed by Kramer (Kramer 2002; Kramer *et al.* 2008) for wood grain patterning. In this model, cells in the cambium responsible for creating the grain pattern orient themselves in response to the flux of auxin, following the lines of auxin flux. Since the orientation of the cells is also included in the calculation of flux, there is a feedback of auxin on its own transport. With this model, Kramer was able to reproduce realistic grain patterns around obstructions such as branch junctions, and the model was able to account for changes in grain patterning in response to wounding (Kramer *et al.* 2008). Further verification was provided by using GC-MS (gas chromatography-mass spectroscopy) to measure auxin gradients in the cambium. However, it was not a cellular model, but rather a continuum model (Kramer 2007) with the discretization used for simulation being larger then single cells. Although at a different level of abstraction, the patterns created by Kramer's model appear qualitatively similar to the laminar flow patterns that can be produced with Mitchison's model if the feedback of flux on the addition of carrier is linear (Feugier *et al.* 2005; Stoma *et al.* 2008). In Kramer's model for wood grain patterning, the polar transport of auxin is restricted to the vector representing what is normally the long (apical–basal) axis of the cells, so that the changing grain orientation can be calculated based on the auxin gradient perpendicular to this vector. To what extent this is equivalent to the flux-based feedback used in the canalization model is unclear; however, it is interesting that the formulation of the model uses auxin concentration to calculate flux.

A TRANSPORT-FEEDBACK MECHANISM FOR PHYLLOTAXIS

Perhaps no other patterning process in plants has been studied more than phyllotaxis. The exquisite patterns of intersecting spirals combined with a curious mathematical connection to Fibonacci numbers and the golden ratio has inspired mathematicians and biologists for over a century (Jean 1994; Adler, Barabe & Jean 1997). Phyllotaxis begins in the shoot apical meristem, a dome-shaped structure at the tip of the shoot. The central zone of the meristem contains a self-sustaining group of undifferentiated stem cells that provide founder cells for all of the aerial structures of the plant (Sussex 1989; Laux 2003). Surrounding the central zone is the peripheral zone, a narrow band of cells with the potential to differentiate and generate new leaf or floral

organs upon receiving the appropriate signal. In order to create highly ordered phyllotactic patterns, organ primordium formation must be tightly controlled, both spatially and temporally (Jean 1994). Thus, a central question in the regulation of phyllotaxis is to understand how the plant knows where and when to position the next primordium at the shoot apex.

The first insight into the regulation of organ positioning at the shoot apex came from Hofmeister (1868) who observed that new organ primordia form periodically, as far as possible from pre-existing ones. This observation led to the inhibition model of phyllotaxis, which postulates that existing organs prevent the formation of new organs in their vicinity. Most models of phyllotaxis are in some way based on this idea, a point echoed by Jönsson *et al*, that phyllotaxis can be explained '. . . by any regular spacing mechanism superimposed on a gradually enlarging generative region' (Jönsson *et al.* 2006). To see how a simple spacing mechanism can lead to the variety of phyllotactic patterns observed in nature, see Douady and Couder (1992) or Smith, Kuhlemeier & Prusinkiewicz (2006b). Yet despite its long research history, the details of the spacing mechanism behind phyllotaxis and its dependence on the polar transport of auxin have only recently come to light. This is perhaps in part because, unlike leaf venation, the problem of how to create regularly spaced spots could be easily explained by diffusing inhibitors (Hellendoorn & Lindenmayer 1974) and reaction–diffusion models (Meinhardt 1982; Meinhardt, Koch & Bernasconi 1998), and thus there was little reason to expect a transport-driven process. However, experimental support for reaction-diffusion or inhibitors in phyllotaxis patterning has not materialized. The wealth of new data about auxin and its transporters point to a transport-feedback mechanism instead (Reinhardt *et al.* 2003; Heisler *et al.* 2005; Reinhardt 2005; Jönsson *et al.* 2006; de Reuille *et al.* 2006; Smith *et al.* 2006a).

The involvement of polar auxin transport in organ formation firstly became evident from experiments where the auxin transport machinery was impaired. Inhibition of auxin movement by chemical treatment or mutation in the auxin exporter PIN1 arrests flower formation without affecting stem growth and meristem maintenance, resulting in a pin-like inflorescence devoid of floral organs (Okada *et al.* 1991; Reinhardt *et al.* 2000, 2003). Exogenous application of a microdroplet of auxin to such pin-formed meristems restores organ formation at the site of micro-application (Reinhardt *et al.* 2000, 2003), demonstrating the role of auxin as an inducer of organogenesis. Using the synthetic auxin responsive promoter DR5, auxin was subsequently shown to accumulate at the sites of organ formation, forming a spot-like pattern that displays a phyllotactic arrangement (Benkova *et al.* 2003; Smith *et al.* 2006a). This pattern appears well ahead of other signs of primordium development, up to several days before physical bulging from the meristem surface. Based on their data, Reinhardt *et al.* (2003) proposed an auxin transport-based model for the regulation of phyllotaxis, suggesting that PIN1 in the surface layer of the meristem directs auxin fluxes to

convergence points which then trigger organogenesis. Bulging primordia inhibit new organ formation in their vicinity – not by emitting an inhibitor molecule – but by draining the activator of organogenesis, auxin, via their initiating midvein. As a result, a spacing mechanism is created by transport, preventing new organs from forming too close to existing ones as observed by Hofmeister (1868).

Given that the formation of auxin maxima is largely controlled by the exporter PIN1, a key question is what determines the orientation of PIN1 within a cell. A hypothesis came from simulation modelling studies (Jönsson *et al.* 2006; Smith *et al.* 2006a), where it was proposed that cells are able to sense the auxin concentration in neighbouring cells, and orient their PIN1 proteins preferentially towards neighbours with higher concentration. As in the case of reaction–diffusion, random fluctuations cause some cells to have slightly higher auxin concentration then others, producing a slight bias of the PIN1 polarization in neighbouring cells. The additional transport caused by this bias increases the cells' concentration of auxin leading to the recruitment of even more PINs in the neighbouring cells. This results in a convergence point of both PIN1 expression and auxin accumulation (local activation), while the draining of auxin from surrounding tissue prevents the formation of other convergence points nearby (longer range inhibition). Thus, the up-the-gradient PIN1 polarization causes a positive feedback loop of auxin on its own transport, and creates regularly spaced peaks in much the same way as reaction-diffusion (Fig. 2B). Implemented on simulation models of a growing shoot meristem, this mechanism was able to create a variety of the phyllotaxis patterns observed in nature (Jönsson *et al.* 2006; Smith *et al.* 2006a). Some support for the existence of an up-gradient polarization mechanism has since been provided by auxin micro-application experiments on tomato vegetative apices (Bayer *et al.* 2009).

It is likely that an up-gradient transport-feedback mechanism is behind other patterning events in plants. The convergence points of auxin that appear in the margin of young Arabidopsis leaves that initiate the bottom portion of the first vein loops are also accompanied by a convergence of PIN1 expression in the margin that is consistent with the up-gradient mechanism (Scarpella *et al.* 2006). Such mechanism may also account for the specification of incipient leaflet primordia in compound leaves (Barkoulas *et al.* 2008).

CAN A SINGLE MECHANISM OF PIN POLARIZATION EXPLAIN BOTH LEAF VENATION AND PHYLLOTAXIS?

Phyllotaxis and leaf venation patterns appear very different, and require mechanisms with different properties. Phyllotaxis requires a mechanism capable of making regularly spaced peaks, and venation requires a mechanism that can create a connected network of strands. However, at the molecular level, these two patterning processes involve the same components. High auxin levels (based on the DR5 data), as well as the upregulation of PIN1, are the earliest

known markers for both processes (Reinhardt *et al.* 2003; Heisler *et al.* 2005; Scarpella *et al.* 2006; Smith *et al.* 2006a). Simulation models suggest that both types of patterns can be created with auxin transport-feedback models, the main difference being the strategy for orienting PIN. The canalization model uses with-the-flux PIN orientation, whereas the phyllotaxis models orient PIN up-the-gradient. This raises the question of how the plant chooses between these two possibilities.

The simplest explanation would be that different tissue types use different strategies for orienting PIN. In the Arabidopsis root, it has been shown that in some cases PIN orientation can be a function of cell type (Vieten *et al.* 2005; Wisniewska *et al.* 2006). When PINs not normally found in certain cell types are expressed ectopically, they can follow the orientation of other PINs normally expressed in that cell type. In this scenario, cells in the surface layer of the shoot apex, or in the margin of the developing leaf would use up-the-gradient PIN1 polarization to form convergence points. In the interior of the shoot apex and the young growing leaf, PINs would polarize with-the-flux and form strands. Figure 6 shows a simulation model based on this idea.

Two recent simulation studies propose that both types of patterns can be explained by a single strategy for PIN polarization. In simulations where PIN production is promoted by auxin, the auxin peaks created by the up-gradient mechanism were not always stable and could move around in the tissue (Heisler & Jönsson 2006). Merks *et al.* (2007) exploited this observation, and suggested that the convergence point of auxin that initiates leaf primordium formation subsequently moves as a travelling wave into inner tissue. In their model, PIN polarity follows this moving peak of auxin, leaving behind a strand of PINs polarized in the direction of the travelling wave. This strand would then differentiate into a vein. Merks *et al.* argued that a mechanism that responds to auxin concentration is more likely, since no molecular mechanism capable of sensing net auxin flux across a membrane section has been found in plants. Mitchison, however, suggested that cells might be able to use the cells' internal auxin concentration gradient to sense flux, an idea shown to be plausible by the simulation models of Kramer (2009).

Stoma *et al.* took the opposite approach and proposed a model to explain phyllotaxis based on the with-the-flux mechanism. In their model, when auxin reaches a threshold concentration in the surface layer of cells in the shoot apex, it initiates a primordium, and auxin is allowed to flow into internal tissue. This flux causes the PINs in neighbouring cells to polarize towards the primordium centre, giving the characteristic PIN1 convergence points around primordia observed experimentally (Reinhardt *et al.* 2003). In internal tissue, flux-based PIN1 orientation causes strands to form as in the canalization model. They proposed that PIN1 responds differently to flux in the surface layer of cells than in the internal tissue of the shoot apex. They show with simulations on sheets of cells that broad PIN1 expression domains can be created when the PIN response to auxin
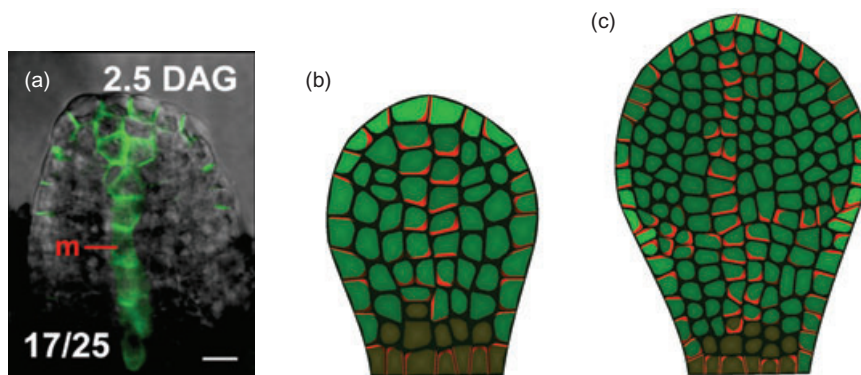
Figure 6. Simulation of early steps in vein formation on a growing leaf. (a) PIN1:GFP picture of a young Arabidopsis leaf showing PIN proteins in margin cells orienting towards the tip to create a convergence point there. A PIN expression domain extends from this convergence point into the interior of the leaf where the midvein (m) will form (image reproduced with permission from Scarpella *et al.* 2006). (b–c) Simulation model of a growing leaf with up-the-gradient PIN orientation in the margin cells and with-the-flux orientation of PIN in interior cells. PIN1 localization at the plasma membrane is shown in red, auxin levels in green. The up-the-gradient mechanism causes self-organizing peaks of auxin appear in the margin. When the auxin at these peaks builds up to a threshold concentration, it leaks into internal cells and the with-the-flux mechanism causes vein strands to appear. As the leaf grows (c), more space is created in the margin allowing more convergence points appear. This in turn causes more veins to be initiated. Dark cells at the bottom are sinks for auxin and represent the existing vasculature of the plant. For simulation equations and parameters see the supplemental materials. Scale bar, 10 μm.

flux is linear, and that canalization occurs with a non-linear response (Mitchison 1980; Feugier *et al.* 2005). This would explain why strands of PIN expression do not appear in the surface layer of cells in the shoot apex.

In an effort to choose between the possibilities, Bayer *et al.* (2009) focused their attention on the early events in the initiation of the midvein. This is the point where the two patterning processes intersect, and through a careful analysis of PIN expression and polarity, they came to the conclusion that both with-the-flux and up-the-gradient polarization must be operating at the same time. When a convergence point that initiates a primordium first appears in the surface layer of the shoot apex, the cells around it are all polarized towards a few cells in its centre. This includes cells in the inner layers of the meristem directly below the convergence point. This is consistent with an up-the-gradient mechanism for PIN1 polarization, as DR5 data (and the orientation of the PINs themselves) suggest that this area is high in auxin. As development proceeds, a strand of cells with high PIN1 expression extends from the convergence point in the surface layer into inner tissue. The PIN1 polarity of the cells in the centre of the convergence point, and those immediately below, switches towards the direction of the extending vein, suggesting a transition to a different polarizing mechanism, such as with-the-flux. Note that this could also occur if the auxin peak were to move, as predicted by the Merks *et al.* (2007) model; however, such movement has not been observed experimentally. In fact, the peak in DR5 reporter expression at the leaf tip gets brighter as leaf development progresses (Mattsson *et al.* 2003; Scarpella *et al.* 2006), likely due to the activation of auxin biosynthesis genes (Schwendener 1878; Cheng *et al.* 2007; Zhao 2008). In addition, the extending midvein is accompanied by strong lateral PIN1 polarization in adjacent cells, pointing towards the central cells in the forming

midvein. Scarpella *et al.* (2006) observed similar PIN1 localization in young leaves, where DR5 reporter expression in the vein occurs concomitantly with PIN1 expression, suggesting that auxin levels are high during these early stages. It is thus difficult to reconcile strong lateral polarization towards the centre of the midvein with a mechanism based on flux alone. These observations led Bayer *et al.* (2009) to propose a combined model in which PINs can transition from polarizing up-the-gradient at low auxin levels, to with-the-flux at higher levels. Simulations of their model reproduced the switch from early apical to basal polarity, as well as strong lateral PIN1 polarity. In addition, their model maintained high auxin levels throughout the simulations, as the peak of auxin in the surface layer was extended to a strand in the forming vein. Interestingly, this lateral PIN1 polarization can also be seen in higher order veins (Fig. 7). Whether the dual model can account for the hierarchy of vein patterning seen in mature leaves remains to be investigated.

A TRANSPORT-FEEDBACK MECHANISM INVOLVED IN LATERAL ROOT INITIATION

Just as the shoot apex is responsible for the specification of the aerial structure of the plant, the root apex creates the plant's subsurface architecture. In Arabidopsis, the root tip is a highly ordered structure, with a network of several members of the PIN protein family directing auxin in a reflux loop about the quiescent centre where the root stem cells reside (Scheres, Benfey & Dolam 2002; Grieneisen *et al.* 2007). This results in an auxin maximum at the centre of the root tip that is thought to be a major player in root apical meristem organization and maintenance (Sabatini *et al.* 1999; Blilou *et al.* 2005; Bansal *et al.* 2006). As in the shoot, the root also produces lateral organs to expand its
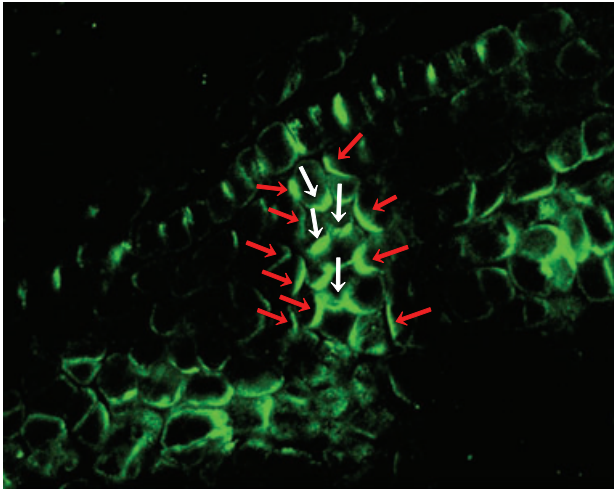
Figure 7. Basal and lateral PIN1 polarization in an initiating second order vein in an Arabidopsis leaf. Immunolocalization of Arabidopsis PIN1 showing basal polarity in the centre of the extending strand (white arrows) with lateral polarization towards the centre (red arrows) at the sides. Figure adapted from Bayer *et al.* (2009).

structure, and experimental data suggest that this process is likewise triggered by elevated auxin levels (Blakely *et al.* 1988; Laskowski *et al.* 1995; Sussex *et al.* 1995; Dubrovsky *et al.* 2008). High DR5 reporter expression in founder cells is currently the earliest known marker for lateral root initiation (Dubrovsky *et al.* 2008). These early peaks in DR5 reporter expression, however, do not appear to be accompanied by major changes in PIN expression or polarity, as is the case with organ formation in the shoot apex. This raises the question as to how cells in the pericycle are able to accumulate auxin preferentially over their neighbours, given that they are in the middle of a basally directed auxin transport stream (Sauer *et al.* 2006).

Noticing that lateral roots often appear on the outside of a curve in the root (Fortin, Pierce & Poff 1989), Laskowski *et al.* (2008) proposed that changes in the shape of cells could affect auxin transport and cause certain cells to preferentially accumulate auxin. Mitchison (1981a,b) had also suggested a geometrical influence on auxin transport involving the position of the vacuole as a possible explanation for gravitropism. Using simulation studies, Laskowski *et al.* showed that under suitable conditions, pericycle cells on the outside of a bend in the root developed higher auxin levels, simply because of the geometry of the cells. Although this mechanism provides a plausible explanation for an initial bias for auxin accumulation, it does not explain why straight roots produce lateral root primordia (Fortin *et al.* 1989) or how auxin accumulation becomes restricted to a few pericycle founder cells.

Laskowski *et al.* propose a transport-feedback mechanism involving auxin importers to supplement the initial bias in auxin accumulation due to cell geometry. Members of the AUX/LAX family have been shown to be important in lateral root initiation (Marchant *et al.* 2002; Swarup *et al.*

2008), and can be upregulated in response to auxin in the root (Laskowski *et al.* 2006, 2008; Paponov *et al.* 2008). Thus, they added auxin-induced production of AUX1 in the pericycle to their model. Cells with slightly higher auxin levels produce more AUX1, causing them to retain even more auxin from the transport stream, thus magnifying any initial bias (local activation). As in the transport-feedback mechanism in the shoot, inhibition of neighbours is caused by a reduction of surrounding auxin levels due to the movement of auxin to the activated cells (long range inhibition). A closely related model was proposed by Lucas *et al.* (2008) whereby polar transport causes auxin to build up in initiation zone of the root until it hits a threshold, which then causes lateral root primordia to initiate. The primordia then consume auxin, reducing the levels in the initiation zone and thus suppressing the formation new ones nearby. Although at a different level of abstraction, it is likely that the transport-feedback mechanism of Laskowski *et al.* based on the upregulation of AUX1 by auxin could provide a molecular basis for the Lucas *et al.* model. A simplified version of this importer driven transport-feedback model is shown in Fig. 2C on a line of cells. In this simulation, the cell geometry was uniform, and the symmetry-breaking initial bias was provided by introducing a small amount of noise to the auxin concentrations. As in the case with the activator-inhibitor system (Fig. 2A) or the up-the-gradient PIN polarization model (Fig. 2B), the mechanism based on the upregulation of AUX1 by auxin is also able to create a pattern of peaks (Fig. 2C).

In contrast to the mechanism based on auxin exporters in the shoot, the transport-feedback model based on importers proposed for the root does not require shifts in transporter polarity. The lateral root primordium founder cells which become activated and express elevated levels of AUX1 distribute the importer uniformly on their plasma membrane (Laskowski *et al.* 2008), and exporter polarities are largely unaffected in the early stages of founder cell specification (Benkova *et al.* 2003). The overall levels of the exporters at the plasma membrane do, however, have an effect on the spacing of lateral root primordia, playing a more passive role in patterning in the root, as seems to be the case with importers in the shoot.

CONCLUSION

Over the last 30 years, experimental evidence has been accumulating that the feedback of auxin on its own transport is responsible for a significant amount of patterning in plants. Simulation studies of transport-feedback mechanisms have demonstrated their plausibility, and in many cases provide the best fit between experimental data and emergent model behaviour. Although auxin transport-feedback models represent a common framework for many patterning events in plants, the means by which auxin feeds back on its own transport appears to vary depending on the developmental event considered. The specification of leaf or floral organ primordia in the shoot apex depends on coordinated changes in exporter polarity, resulting in a

spacing mechanism that can create phyllotactic patterns. Lateral root primordium initiation is often much more irregular than organ initiation in the shoot, but nevertheless still requires a spacing mechanism. A transport-feedback model based on the upregulation of importers by auxin has recently been proposed, which does not appear to require changes in transporter polarity. Leaf venation, which has the completely different patterning requirement to create a connected network of strands, is also best explained by a transport-feedback process. Using the same molecular machinery as the mechanism proposed for phyllotaxis, a simple change in the strategy for auxin exporter polarization, from up-the-gradient to with-the-flux, can switch the outcome of the mechanism from the production of uniformly spaced spots to a system of connected strands.

It is unclear as to whether transport-feedback mechanisms can create as wide a variety of patterns as has been possible with reaction–diffusion. By using multiple substances and interactions, reaction–diffusion has been able to reproduce many patterns observed in biology. With auxin-based patterning, it seems hard to imagine that such a variety of patterning events can be controlled by one hormone. However, transport is a much more sophisticated means of movement than diffusion, and the diversity of methods by which it can be controlled may provide a clue as to how auxin can do so many different things.

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REFERENCES

Adler I., Barabe D. & Jean R.V. (1997) A history of the study of phyllotaxis. *Annals of Botany* **80**, 231–244.

Bainbridge K., Guyomarc'h S., Bayer E., Swarup R., Bennett M., Mandel T. & Kuhlemeier C. (2008) Auxin influx carriers stabilize phyllotactic patterning. *Genes & Development* **22**, 810–823.

Bansal M.G., Della G. & di Bernardo D. (2006) Inference of gene regulatory networks and compound mode of action from time course gene expression profiles. *Bioinformatics* **22**, 815–822.

Barkoulas M., Hay A., Kougioumoutzi E. & Tsiantis M. (2008) A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. *Nature Genetics* **40**, 1136–1141.

Bayer E.M., Smith R.S., Mandel T., Nakayama N., Sauer M., Prusinkiewicz P. & Kuhlemeier C. (2009) Integration of transport-based models for phyllotaxis and midvein formation. *Genes & Development* **23**, 373–384.

Benkova E., Michniewicz M., Sauer M., Teichmann T., Seifertova D., Jurgens G. & Friml J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591–602.

Blakely L.M., Blakely R.M., Colowit P.M. & Elliott D.S. (1988) Experimental studies on lateral root-formation in radish seedling roots. 2. Analysis of the dose-response to exogenous auxin. *Plant Physiology* **87**, 414–419.

Blilou I., Xu J., Wildwater M., Willemsen V., Paponov I., Friml J., Heidstra R., Aida M., Palme K. & Scheres B. (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* **433**, 39–44.

Bouyer D., Geier F., Kragler F., Schnittger A., Pesch M., Wester K., Balkunde R., Timmer J., Fleck C. & Hülskamp M. (2008) Two-dimensional patterning by a trapping/depletion mechanism: the role of TTG1 and GL3 in Arabidopsis trichome formation. *PLoS Biol* **6**, e141.

Casimiro I., Marchant A., Bhalerao R.P., et al. (2001) Auxin transport promotes Arabidopsis lateral root initiation. *The Plant Cell* **13**, 843–852.

Cheng Y., Dai X. & Zhao Y. (2007) Auxin Synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. *The Plant Cell* **19**, 2430–2439.

De Smet I., Tetsumura T., De Rybel B., et al. (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. *Development* **134**, 681–690.

Deyholos M.K., Cordner G., Beebe D. & Sieburth L.E. (2000) The SCARFACE gene is required for cotyledon and leaf vein patterning. *Development* **127**, 3205–3213.

Dhonukshe P., Aniento F., Hwang I., Robinson D.G., Mravec J., Stierhof Y.D. & Friml J. (2007) Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in Arabidopsis. *Current Biology* **17**, 520–527.

Dhonukshe P., Tanaka H., Goh T., et al. (2008) Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. *Nature* **456**, 962–975.

Digiuni S., Schellmann S., Geier F., et al. (2008) A competitive complex formation mechanism underlies trichome patterning on Arabidopsis leaves. *Molecular Systems Biology* **4**, Article No. 217.

Ditengou F.A., Tealea W.D., Kochersperger P., et al. (2008) Mechanical induction of lateral root initiation in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 18818–18823.

Douady S. & Couder Y. (1992) Phyllotaxis as a physical self-organized growth process. *Physical Review Letters* **68**, 2098–2101.

Dubrovsky J.G., Sauer M., Napsucialy-Mendivil S., Ivanchenko M.G., Friml J., Shishkova S., Celenza J. & Benková E. (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 8790–8794.

Esmon C.A., Tinsley A.G., Ljung K., Sandberg G., Hearne L.B. & Liscum E. (2006) A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 236–241.

Feugier F.G. & Iwasa Y. (2006) How canalization can make loops: a new model of reticulated leaf vascular pattern formation. *Journal of Theoretical Biology* **243**, 235–244.

Feugier F.G., Mochizuki A. & Iwasa Y. (2005) Self-organization of the vascular system in plant leaves: inter-dependent dynamics of auxin flux and carrier proteins. *Journal of Theoretical Biology* **236**, 366–375.

Fortin M.C., Pierce F.J. & Poff K.L. (1989) The pattern of secondary root-formation in curving roots of Arabidopsis-Thaliana (L) Heynh. *Plant, Cell & Environment* **12**, 337–339.

Friml J., Wisniewska J., Benkova E., Mendgen K. & Palme K. (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature* **415**, 806–809.

Friml J., Vieten A., Sauer M., Weijers D., Schwarz H., Hamann T., Offringa R. & Jurgens G. (2003) Efflux-dependent auxin

gradients establish the apical-basal axis of Arabidopsis. *Nature* **426,** 147–153.

Fujita H. & Mochizuki A. (2006a) The origin of the diversity of leaf venation pattern. *Developmental Dynamics* **235,** 2710–2721.

Fujita H. & Mochizuki A. (2006b) Pattern formation of leaf veins by the positive feedback regulation between auxin flow and auxin efflux carrier. *Journal of Theoretical Biology* **241,** 541–551.

Galweiler L., Guan C., Muller A., Wisman E., Mendgen K., Yephremov A. & Palme K. (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* **282,** 2226–2230.

Geldner N., Friml J., Stierhof Y.D., Jurgens G. & Palme K. (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413,** 425–428.

Geldner N., Anders N., Wolters H., Keicher J., Kornberger W., Muller P., Delbarre A., Ueda T., Nakano A. & Jurgens G. (2003) The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* **112,** 219–230.

Gierer A. & Meinhardt H. (1972) A theory of biological pattern formation. *Kybernetik* **12,** 30–39.

Goldsmith M.H. (1966) Movement of indoleacetic acid in coleoptiles of Avena sativa L. II. Suspension of polarity by total inhibition of the basipetal transport. *Plant Physiology* **41,** 15–27.

Goldsmith M.H.M. & Goldsmith T.H. (1981) Quantitative predictions for the chemiosmotic uptake of Auxin. *Planta* **153,** 25–33.

Goldsmith M.H.M., Goldsmith T.H. & Martin M.H. (1981) Mathematical-analysis of the chemosmotic polar diffusion of auxin through plant-tissues. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* **78,** 976–980.

Grieneisen V.A., Xu J., Marée A.F.M., Hogeweg P. & Scheres B. (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* **449,** 1008–1013.

Hardtke C.S. & Berleth T. (1998) The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO Journal* **17,** 1405–1411.

Heisler M.G. & Jönsson H. (2006) Modeling auxin transport and plant development. *Journal of Plant Growth Regulation* **25,** 302–312.

Heisler M.G., Ohno C., Das P., Sieber P., Reddy G.V., Long J.A. & Meyerowitz E.M. (2005) Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. *Current Biology* **15,** 1899–1911.

Hellendoorn P.H. & Lindenmayer A. (1974) Phyllotaxis in Bryophyllum tubiflorum: morphogenetic studies and computer simulations. *Acta Botanica Néerlandica* **23,** 473–492.

Hobbie L., McGovern M., Hurwitz L.R., Pierro A., Liu N.Y., Bandyopadhyay A. & Estelle M. (2000) The axr6 mutants of Arabidopsis thaliana define a gene involved in auxin response and early development. *Development* **127,** 23–32.

Hofmeister W. (1868) *Handbuch der Physiologishen Botanik*. Engelmann, Leipzig, Germany.

Jean R.V. (1994) *Phyllotaxis: A Systematic Study in Plant Morphogenesis*. Cambridge University Press, Cambridge, UK.

Jenik P.D., Gillmor C.S. & Lukowitz W. (2007) Embryonic patterning in Arabidopsis thaliana. *Annual Review of Cell and Developmental Biology* **23,** 207–236.

Jönsson H., Heisler M., Reddy G.V., Agrawal V., Gor V., Shapiro B.E., Mjolsness E. & Meyerowitz E.M. (2005) Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem. *Bioinformatics* **21** (Suppl. 1), i232–i240.

Jönsson H., Heisler M., Shapiro B.E., Meyerowitz E.M. & Mjolsness E. (2006) An auxin-driven polarized transport model for phyllotaxis. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 1633–1638.

Kleine-Vehn J., Dhonukshe P., Sauer M., Brewer P.B., Wiśniewska J., Paciorek T., Benková E. & Friml J. (2008) ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in Arabidopsis. *Current Biology* **18,** 526–531.

Kramer E.M. (2002) A mathematical model of pattern formation in the vascular cambium of trees. *Journal of Theoretical Biology* **216,** 147–158.

Kramer E.M. (2004) PIN and AUX/LAX proteins: their role in auxin accumulation. *Trends in Plant Science* **9,** 578–582.

Kramer E.M. (2007) Computer models of auxin transport: a review and commentary. *Journal of Experimental Botany* **59,** 45–53.

Kramer E.M. (2009) Auxin-regulated cell polarity: an inside job? *Trends in Plant Science* **14,** 242–247.

Kramer E.M., Lewandowski M., Beri S., Bernard J., Borkowski M., Borkowski M.H., Burchfield L.A., Mathisen B. & Normanly J. (2008) Auxin gradients are associated with polarity changes in trees. *Science* **320,** 1610–1610.

Kuhlemeier C. (2007) Phyllotaxis. *Trends in Plant Science* **12,** 143–150.

Laskowski M.J., Williams M.E., Nusbaum H.C. & Sussex I.M. (1995) Formation of lateral root-meristems is a 2-stage process. *Development* **121,** 3303–3310.

Laskowski M., Biller S., Stanley K., Kajstura T. & Prusty R. (2006) Expression profiling of auxin-treated Arabidopsis roots: toward a molecular analysis of lateral root emergence. *Plant and Cell Physiology* **47,** 788–792.

Laskowski M., Grieneisen V., Hofhuis H., ten Hove C.A., Hogeweg P., Marée A.F.M. & Scheres B. (2008) Root system architecture from coupling cell shape to auxin transport. *PLoS Biology* **6,** e307.

Laux T. (2003) The stem cell concept in plants: a matter of debate. *Cell* **113,** 281–283.

Leopold A.C. & Hall O.F. (1966) Mathematical model of polar auxin transport. *Plant Physiology* **41,** 1476–1480.

Leyser O. (2005) Auxin distribution and plant pattern formation: how many angels can dance on the point of PIN? *Cell* **121,** 819–822.

Lucas M., Guédon Y., Jay-Allemand C., Godin C. & Laplaze L. (2008) An auxin transport-based model of root branching in Arabidopsis thaliana. *PLoS ONE* **3,** e3673.

Marchant A., Kargul J., May S.T., Muller P., Delbarre A., Perrot-Rechenmann C. & Bennett M.J. (1999) AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *EMBO Journal* **18,** 2066–2073.

Marchant A., Bhalerao R., Casimiro I., Eklof J., Casero P.J., Bennett M. & Sandberg G. (2002) AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *The Plant Cell* **14,** 589–597.

Mattsson J., Sung Z.R. & Berleth T. (1999) Responses of plant vascular systems to auxin transport inhibition. *Development* **126,** 2979–2991.

Mattsson J., Ckurshumova W. & Berleth T. (2003) Auxin signaling in Arabidopsis leaf vascular development. *Plant Physiology* **131,** 1327–1339.

Meinhardt H. (1982) *Models of Biological Pattern Formation*. Academic Press, London, UK.

Meinhardt H. (1995) *The Algorithmic Beauty of Sea Shells*. Springer-Verlag Inc., New York, NY, USA.

Meinhardt H.J., Koch A. & Bernasconi G. (1998) Models of pattern formation applied to plant development. In *Symmetry in Plants* (eds D. Barabe & R. V. Jean) pp. 723–758. World Scientific Publishing, Singapore, Singapore.

Merks R.M.H., Van de Peer Y., Inze D. & Beemster G.T.S. (2007)

Canalization without flux sensors: a traveling-wave hypothesis. *Trends in Plant Science* **12,** 384–390.

Mitchison G.J. (1980) A model for vein formation in higher plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **207,** 79–109.

Mitchison G.J. (1981a) The effect of intracellular geometry on auxin transport I. Centrifugation experiments. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **214,** 53–67.

Mitchison G.J. (1981b) The effect of intracellular geometry on auxin transport II. Geotropism in shoots. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **214,** 69–83.

Mitchison G.J. (1981c) The polar transport of auxin and vein patterns in plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **295,** 461–271.

Murray J.D. (2002) *Mathematical Biology I.* Springer-Verlag, Berlin, Germany.

Okada K., Ueda J., Komaki M.K., Bell C.J. & Shimura Y. (1991) Requirement of the auxin polar transport-system in early stages of Arabidopsis floral bud formation. *The Plant Cell* **3,** 677–684.

Ongaro V. & Leyser O. (2008) Hormonal control of shoot branching. *Journal of Experimental Botany* **59,** 67–74.

Paciorek T., Zazimalova E., Ruthardt N., *et al.* (2005) Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* **435,** 1251–1256.

Paponov I.A., Paponov M., Teale W., Menges M., Chakrabortee S., Murray J.A.H. & Palme K. (2008) Comprehensive transcriptome analysis of auxin responses in Arabidopsis. *Molecular Plant* **1,** 321–337.

Przemeck G.K.H., Mattsson J., Hardtke C.S., Sung Z.R. & Berleth T. (1996) Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization. *Planta* **200,** 229–237.

Raven J.A. (1975) Transport of indolacetic acid in plant cells in relation to pH and electrical gradients, and its significance for polar IAA transport. *New Phytologist* **74,** 163–172.

Reinhardt D. (2005) Phyllotaxis: a new chapter in an old tale about beauty and magic numbers. *Current Opinion in Plant Biology* **8,** 487–493.

Reinhardt D., Mandel T. & Kuhlemeier C. (2000) Auxin regulates the initiation and radial position of plant lateral organs. *The Plant Cell* **12,** 507–518.

Reinhardt D., Pesce E.-R., Stieger P., Mandel T., Baltensperger K., Bennett M., Traas J., Friml J. & Kuhlemeier C. (2003) Regulation of phyllotaxis by polar auxin transport. *Nature* **426,** 255–260.

de Reuille P.B., Bohn-Courseau I., Ljung K., Morin H., Carraro N., Godin C. & Traas J. (2006) Computer simulations reveal properties of the cell–cell signaling network at the shoot apex in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 1627–1632.

Rolland-Lagan A.G. & Prusinkiewicz P. (2005) Reviewing models of auxin canalization in the context of leaf vein pattern formation in Arabidopsis. *The Plant Journal* **44,** 854–865.

Rubery P.H. & Sheldrake A.R. (1974) Carrier-mediated auxin transport. *Planta* **118,** 101–121.

Runions A., Fuhrer M., Lane B., Federl P., Rolland-Lagan A.G. & Prusinkiewicz P. (2005) Modeling and visualization of leaf venation patterns. *Acm Transactions on Graphics* **24,** 702–711.

Sabatini S., Beis D., Wolkenfelt H., *et al.* (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* **99,** 463–472.

Sachs T. (1969) Polarity and induction of organized vascular tissues. *Annals of Botany* **33,** 263–275.

Sachs T. (1975) Induction of transport channels by auxin. *Planta* **127,** 201–206.

Sachs T. (1981) The control of patterned differentiation of vascular tissues. *Advances in Botanical Research* **9,** 151–262.

Sachs T. & Cohen D. (1982) Circular vessels and the control of vascular differentiation in plants. *Differentiation* **21,** 22–26.

Sauer M., Balla J., Luschnig C., Wisniewska J., Reinöhl V., Friml J. & Benková E. (2006) Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes & Development* **20,** 2902–2911.

Scarpella E. & Meijer A.H. (2004) Pattern formation in the vascular system of monocot and dicot plant species. *New Phytologist* **164,** 209–242.

Scarpella E., Francis P. & Berleth T. (2004) Stage-specific markers define early steps of procambium development in Arabidopsis leaves and correlate termination of vein formation with mesophyll differentiation. *Development* **131,** 3445–3455.

Scarpella E., Marcos D., Friml J. & Berleth T. (2006) Control of leaf vascular patterning by polar auxin transport. *Genes & Development* **20,** 1015–1027.

Scheres B., Benfey P. & Dolam L. (2002) Root development. In *The Arabidopsis Book* (eds E.M. Somerville & C.R. Meyerowitz). American Society of Plant Biologists, Rockville, MD, USA.

Schwendener S. (1878) *Mechanische Theorie der Blattstellungen.* Engelmann, Leipzig, Germany.

Sick S., Reinker S., Timmer J. & Schlake T. (2006) WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. *Science* **314,** 1447–1450.

Sieburth L.E. (1999) Auxin is required for leaf vein pattern in Arabidopsis. *Plant Physiology* **121,** 1179–1190.

Sieburth L.E., Muday G.K., King E.J., Benton G., Kim S., Metcalf K.E., Meyers L., Seamen E. & Van Norman J.M. (2006) SCARFACE encodes an ARF-GAP that is required for normal auxin efflux and vein patterning in Arabidopsis. *The Plant Cell* **18,** 1396–1411.

Smith R.S. (2008) The role of auxin transport in plant patterning mechanisms. *PLoS Biology* **6,** e323.

Smith R.S., Guyomarc'h S., Mandel T., Reinhardt D., Kuhlemeier C. & Prusinkiewicz P. (2006a) A plausible model of phyllotaxis. *Proceedings of the National Academy of Sciences of the United States of America* **103,** 1301–1306.

Smith R.S., Kuhlemeier C. & Prusinkiewicz P. (2006b) Inhibition fields for phyllotactic pattern formation: a simulation study. *Canadian Journal of Botany* **84,** 1635–1649.

Steinmann T., Geldner N., Grebe M., Mangold S., Jackson C.L., Paris S., Galweiler L., Palme K. & Jurgens G. (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. *Science* **286,** 316–318.

Stoma S., Lucas M., Chopard J., Schaedel J., Traas J. & Godin C. (2008) Flux-based transport enhancement as a plausible unifying mechanism for auxin transport in meristem development. *PLoS Computational Biology* **4,** e1000207.

Sussex I.M. (1989) Developmental programming of the shoot meristem. *Cell* **56,** 225–229.

Sussex I.M., Godoy J.A., Kerk N.M., Laskowski M.J., Nusbaum H.C., Welsch J.A. & Williams M.E. (1995) Cellular and molecular events in a newly organizing lateral root-meristem. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **350,** 39–43.

Swarup K., Benkova E., Swarup R., *et al.* (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology* **10,** 946–954.

Swarup R., Kramer E.M., Perry P., Knox K., Leyser H.M.O., Haseloff J., Beemster G.T.S., Bhalerao R. & Bennett M.J. (2005) Root gravitropism requires lateral root cap and epidermal cells

for transport and response to a mobile auxin signal. *Nature Cell Biology* **7,** 1057–1065.

Takebe I. (1971) Regeneration of whole plants from isolated mesophyll protoplasts of tobacco. *Naturwissenschaften* **58,** 318–320.

Tanaka H., Dhonukshe P., Brewer P.B. & Friml J. (2006) Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. *Cellular and Molecular Life Sciences* **63,** 2738–2754.

Tatematsu K., Kumagai S., Muto H., Sato A., Watahiki M.K., Harper R.M., Liscum E. & Yamamoto K.T. (2004) MASSUGU2 encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in Arabidopsis thaliana. *The Plant Cell* **16,** 379–393.

Turing A.M. (1952) The chemical basis of morphogenesis. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences* **237,** 37–52.

Vieten A., Vanneste S., Wisniewska J., Benkova E., Benjamins R., Beeckman T., Luschnig C. & Friml J. (2005) Functional redundancy of PIN proteins is accompanied by auxindependent cross-regulation of PIN expression. *Development* **132,** 4521–4531.

Weijers D., Sauer M., Meurette O., Friml J., Ljung K., Sandberg G., Hooykaas P. & Offringa R. (2005) Maintenance of embryonic auxin distribution for apical-basal patterning by PIN-FORMED-dependent auxin transport in Arabidopsis. *The Plant Cell* **17,** 2517–2526.

Weijers D., Schlereth A., Ehrismann J.S., Schwank G., Kientz M. & Jurgens G. (2006) Auxin triggers transient local signaling for cell specification in Arabidopsis embryogenesis. *Developmental Cell* **10,** 265–270.

Wisniewska J., Xu J., Seifertova D., Brewer P.B., Ruzicka K., Blilou I., Rouquie D., Scheres B. & Friml J. (2006) Polar PIN localization directs auxin flow in plants. *Science* **312,** 883.

Wolpert L. (1969) Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25,** 1–47.

Zhao Y. (2008) The role of local biosynthesis of auxin and cytokinin in plant development. *Current Opinion in Plant Biology* **11,** 16–22.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix 1. Supplemental materials – simulation details.

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